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THE UNITED STATES OF AMERICA

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RELATED PCT APPLICATION NUMBER: *PCT/US04/42007*

UNITED STATES PATENT AND TRADEMARK OFFICE
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Jon W Dudas

Under Secretary
of Commerce for Intellectual Property
and Acting Director of the
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Provisional Application Cover Sheet

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This is a request for filing a PROVISIONAL APPLICATION under 37 C.F.R. § 1.53(b)(2).

Docket Number: Q3325	Type a plus sign (+) inside this box	+

Inventor(s)/Applicant(s)			
Last Name	First Name	Middle Initial	Residence (City and either State or Foreign Country)
Diamond Gosalia	Scott Dhaval		Bala Cynwyd, PA Philadelphia, PA

Title of the Invention (280 Characters Maximum)

Methods and Devices for Running Reactions on a Target Plate for MALDI Mass Spectrometry

Correspondence Address

University of Pennsylvania
Center For Technology Transfer
3160 Chestnut Street
Suite 200

City: Philadelphia State: Pennsylvania Zip Code: 19104 - 6283 Country: US

Enclosed Application Parts (*check all that apply*)

[X] Specification *Number of pages*: 22 [ ] Small Entity Statement

[ ] Drawing(s) *Number of sheets*: [ ] Other (specify)

Method of Payment (*check one*)

[ ] Our Check No. ____ is enclosed to cover the Provisional filing fees. A duplicate copy of this sheet is enclosed. [X] The Commissioner is hereby authorized to charge filing fees and credit Deposit Account No. 13-2469. A duplicate copy of this sheet is enclosed. [ ] Payment by credit card. Form PTO-2038 is attached.	Provisional Filing Fee Amount ($)	$ 80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[ ] No
[ ] Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,

Signature: Scott L Diamond Date: 12-12-03

Typed or Printed Name: Scott Diamond

[ ] Additional inventors are being named on separately numbered sheets attached hereto.

PROVISIONAL APPLICATION FILING ONLY

PROVISIONAL APPLICATION SUBMISSION TO USPTO – CONTENTS PAGE

Penn Docket Number : Q3325

First-named Inventor : Diamond

Submission Date : 12/15/03

Prepared by : Matt Thomas

CONTENTS LISTED IN ORDER :

Total Number of Pages : 22

TITLE: **Methods and Devices for running reactions on a target plate for MALDI mass spectrometry**

INVENTORS: Scott L. Diamond and Dhaval Gosalia
Institute for Medicine and Engineering
University of Pennsylvania

INVENTION DATE: Friday, September 19, 2003

DISCLOSURE DATE: Monday, September 21, 2003

The method allows the ability to run individual nanoliter to microliter volume liquid reactions at positions on a mass spectrometry (MS) target plate ("target"). The target is a metal surface that is flat or has wells or has coatings or grooves to maintain sample position. Additional constituents are added to each reaction by various liquid handling protocols to initiate reactions. Finally, the constituents and products of each reaction volume are prepared by vacuum drying and aerosol deposition of MALDI matrix followed by mass spectrometry without the need to remove the sample from the MS target for various typical manipulations (desalting, extraction, digestion, MALDI matrix addition and formation). The technology has application towards reaction optimization, high throughput drug discovery, high throughput drug selectivity profiling or toxicity testing. A range from 100 to 1000 reaction samples per square centimeter of target can be achieved. Reaction volumes can range from 1 nanoliter to 2000 nanoliters.

NOVEL FEATURES

1) Method for running biological reactions on a MS target followed by MALDI mass spectrometry analysis of the samples on the target without the need to remove the sample from the target for desalting, solvent exchange, digestion, matrix addition, matrix formation.

2) Ability to conduct high throughput drug testing on a MALDI MS target.

3) Method and machine to create outstanding quality matrix for MALDI mass spectrometry.

4) Ability to run 100 to 1000 samples per square centimeter of MALDI target.

5) Allows unique opportunities to conduct liquid chromatography (LC) MALDI MS for improved sample throughput.

BACKGROUND

Proteomics and high throughput screening (HTS) are activities that involve the analysis of hundreds to millions of samples. In drug screening, reactions are run in well-plates to produce optical signals (fluorescence, luminescence) indicating that a hit was identified. The search for "label free" drug screening could rely on matrix assisted laser desorption ionization (MALDI) mass spectrometry (MS) which has excellent throughput. However, the reaction constituents would have to be prepared for MS which involves desalting the sample and adding matrix to each sample and then placing each sample on the target. These liquid handling steps are time consuming and expensive and limit the use of MALDI for HTS. The need to conduct HTS on a MALDI target would meet industry demands for label-free HTS with high capacity (10K to 100K screens per day). The disclosed technology allows HTS reactions to be run on a MALDI plate followed by MALDI MS analysis with a detection limit of 1 femtomole of a peptide in 50 nanoliters of sample. This disclosure also allows the power of contact pin printers to load MALDI plates at up to 1000 spots per square centimeter. MALDI matrix can be applied with such high quality that ultrasmall liquid samples can be used while still generating outstanding crystals for the MALDI process to allow ultrasensitive detections.

In proteomics, complex biological samples are separated by 2D electrophoresis and gel plugs are desalted and digested through a throughput-limiting process requiring expensive liquid handling. These steps limit the throughput of proteomics facilities. To avoid the problems of 2D electrophoresis, labs have used liquid chromatography and capillary electrophoresis. The described technology allows the effluent of LC or CE to be placed on the plate for subsequent coating with MALDI matrix.

Competing Technology

We are not aware of prior examples of running biological reactions on a MALDI plate.

We are not aware of prior examples of creating a MALDI matrix by aerosol deposition.

We are aware of no device on the market for aerosol deposition of MALDI matrix onto a target.

What is CLAIMED:

1) A metal target on which individual liquid or solid reactants are placed at unique positions on a MS target by liquid handling (pin printing, piezo printing, positive displacement liquid handling etc.) at volumes ranging from 1 nanoliter to 2000 nanoliters.

2) A target on which subsequent reagents (1 picoliter to 2000 nanoliters) are delivered to each position on the MS target as described in Claim 1 and individual chemical reactions are allowed to proceed.

3) Where the reaction of Claim 2 liquid constituents of the reaction (solvents) are removed by air drying or vacuum drying to leave a defined position of deposition of caked materials.

4) Where MALDI matrix ("matrix") is coated onto the dry target by spray deposition without the need for micropositioning of matrix delivery to each individual sample and whereby the samples do not cross contaminate each other due to the discontinuous nature of an aerosol and the spacing between the samples.

5) Where the MALDI matrix of Claim 4 is a thin film achieved by aerosol deposition to a coating of less than 50 microns by delivery of no more than 10 microliters of MALDI matrix solution delivered to more than 5 sq. cm. of target surface.

6) Where the MALDI matrix of Claim 4 contains volatile solvents such as water and acetonitrile and a supersaturated concentration of MALDI matrix.

7) Where the dry MALDI matrix coating is humidified to achieve outstanding crystals for MALDI mass spectrometry with concomitant mixing of the constituents of the reaction sample into the MALDI matrix.

8) Where the MALDI matrix formed in Claim 7 allows detection of peptides and modified peptides at concentrations in the range of 1 to 50 femtomole of peptide in a 1 to 100 nanoliter sample.

9) Where mobile ions (salt) diffuse from the reaction into surrounding matrix formed by the Method of Claim 7 thereby reducing there concentration by radial diffusion away from the original sample position.

10) Where feature size of the biological reaction ranges of Claim 1 and Claim 2 are from 200 to 1000 microns in diameter by use of contact printing or positive-displacement liquid handling.

11) Whereby the processed "target" is subjected to MALDI MS for analysis of reactants and products formed by reaction at each position by negative or positive mode and by linear or reflectron mode.

12) Where the components of Claim 1 are enzymes, peptides, proteins, DNA, RNA, nucleotides, amino acids, and substrates, catalysts, salts, buffers, and cofactors.

13) Where the components of Claim 2 are enzymes, peptides, proteins, DNA, RNA, nucleotides, amino acids, and substrates, catalysts, salts, buffers, and cofactors.

14) Where the reactions formed in Claim 2 are at high densities of ranging form 10 to 1000 reactions per square centimeter of the target.

15) Where the reactions formed in Claim 2 are useful for detecting drug like activity in a high throughput drug discovery process.

16) Where the reactions formed in Claim 2 are useful for determining the interaction of a drug with many other proteins such as kinases, P450 enyzmes, phosphatases, proteases, etc.

17) A device and its operation for spraying MALDI Matrix on a target where the target is moved at 0.5 to 2 inches per sec and the matrix solution is aerosolized with an ultrasonic nozzle (0.5 to 1 W) or spray nozzle with carrier gas at a flowrate of 0.5 to 2 microliters per minute. Several coatings can be achieved for highly controlled film thickness of the MALDI Matrix.

RELATED TO PRIOR PATENT APPLICATIONS BY DIAMOND

17) Where the subcomponents of each reaction (enzymes, catalysts, buffers) are positioned on a flat target in 1 to 100 nanoliter droplets of a nonvolatile liquid such as glycerol by the use of contact printing.

18) Where the subcomponents of each reaction of Claim 1 are stored on the surface of the target in a controlled environment and can be shipped to alternate locations for use at later times.

19) Where the subcomponents of Claim 2 to be added to the reaction are delivered by aerosol deposition.

20) Where the target comes pre-prepared with numerous enzymes and prepared reaction components for distribution to other users who activate the target and use the methods of the described technology to run the target in their MS machine.

Applications:

1) On-target digestion of proteins for MALDI
2) On-target triggering of bioreactions by addition of a limiting reagent.
3) Highthroughput proteomics
4) Highthroughput reaction optimization

Potential Licence Partners (nonexclusive)

1) Reaction Biology Corporation
2) ABI
3) Waters
4) Perkin Elmer
5) Bruker Daltronics
6) various proteomics and Mass spectrometry companies.
7) Genomic Solutions

Figure 1

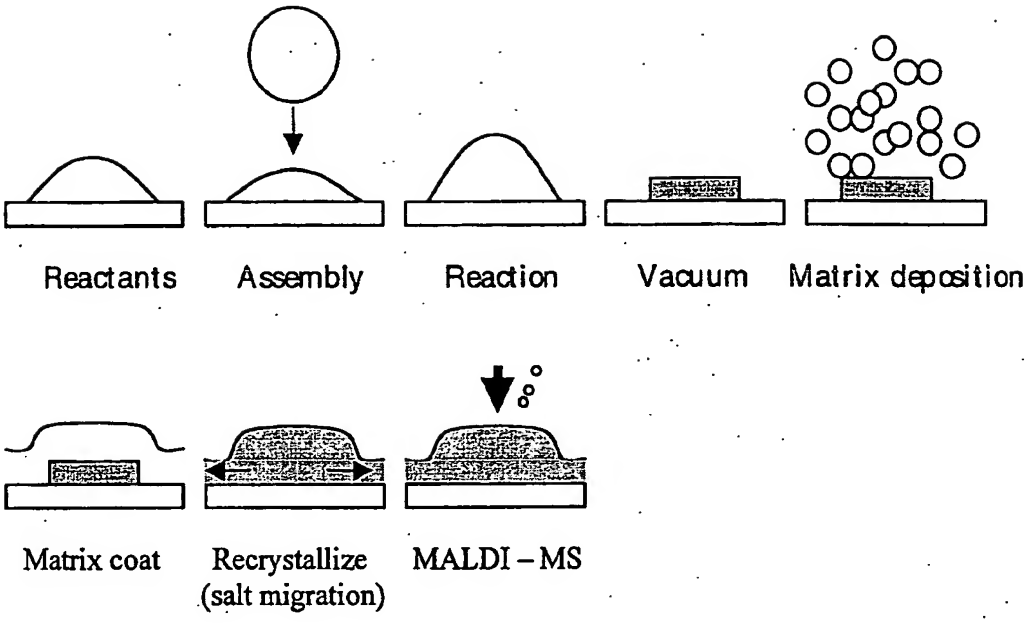

Reactants
Assembly
Reaction
Vacuum
Matrix deposition
Matrix coat
Recrystallize
(salt migration)
MALDI – MS

FIGURE 2

A series of Mass Spectrum for control fibrinogen and on-target treated fibrinogen activated with thrombin (10 U/ml) in 50 nanoliter reactions.

Concentrations were:	18 mg/ml	52.8 micromolar
	9 mg/ml	26.4 micromolar
	4.5 mg/ml	13.2 micromolar
	3 mg/ml	8.8 micromolar
	1.5 mg/ml	4.4 micromolar
	0.75 mg/ml	2.2 micromolar

Spectrum Peaks

Fibrinogen (no reaction)	1264.19 m/z (contamination)
Fibrinogen + thrombin	1539.64 m/z Fibrinopeptide A (calculated 1536.6) 1555.74 m/z Fibrinopeptide B (calculated1569.60

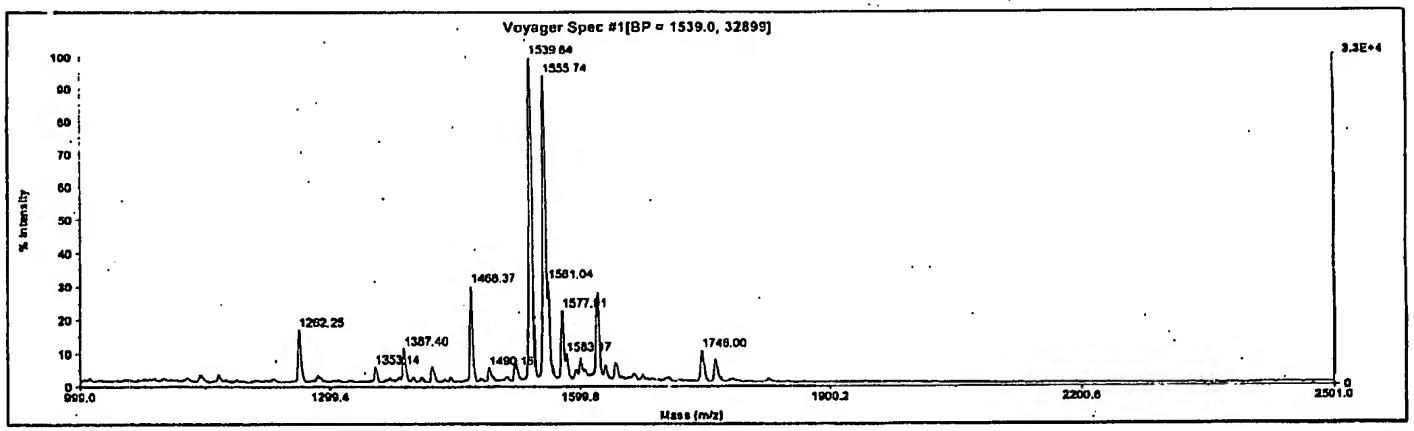
Reaction

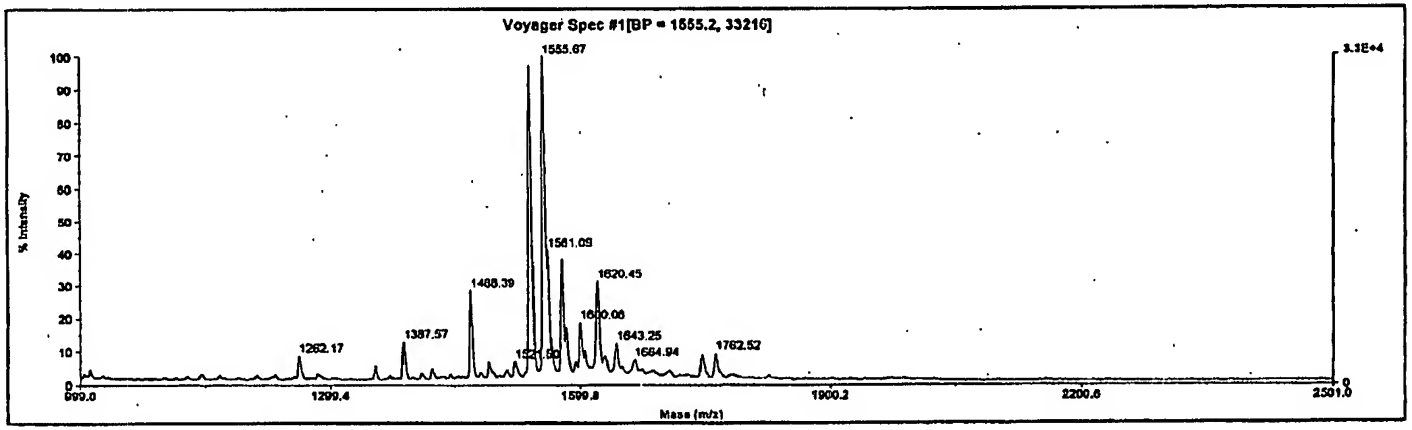
Fibrinogen + thrombin -> fibrin monomer + 2 Fibinopeptide A + 2 Fibrinopeptide B

EXAMPLES

Fibirinogen 18mg/ml

Fibrinopeptide A = 1536
Fibrinopeptide B = 1568

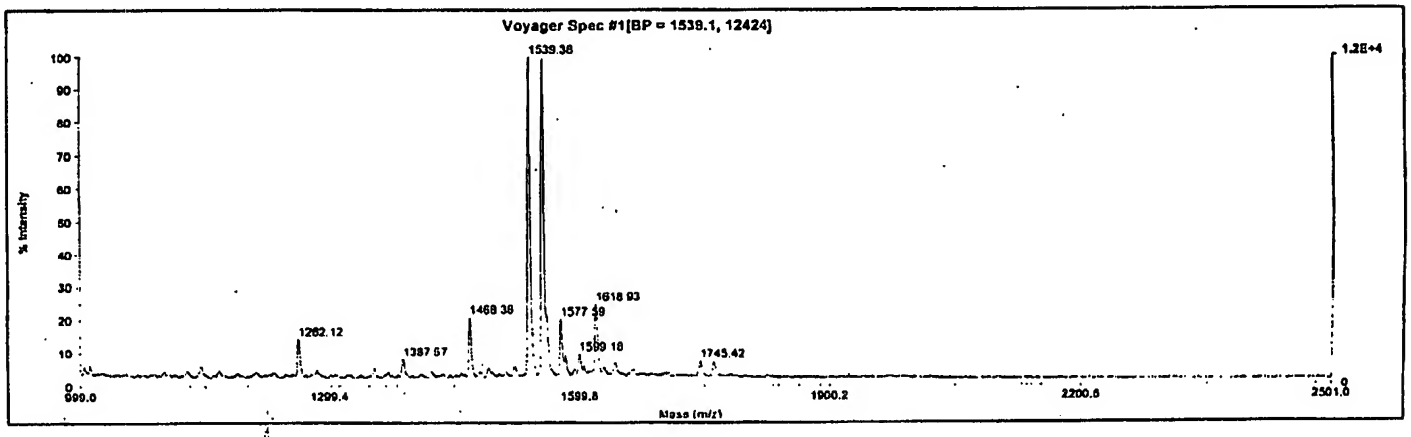




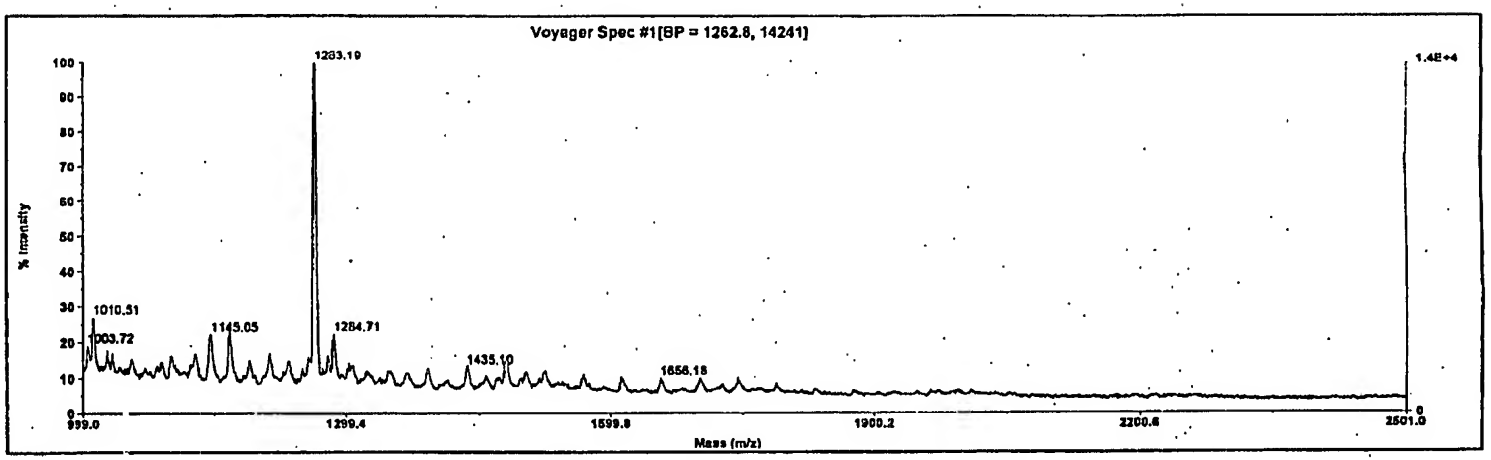
Laser power
Same for all
Conc. = 1800

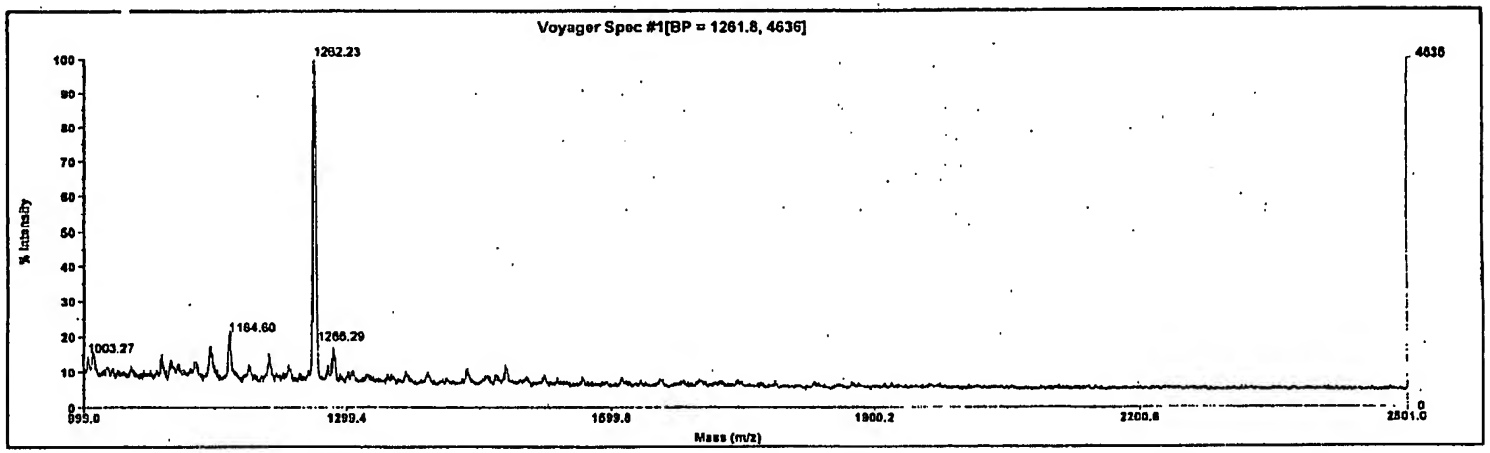
Matrix sprayed
Sinapic acid

Linear positive mode

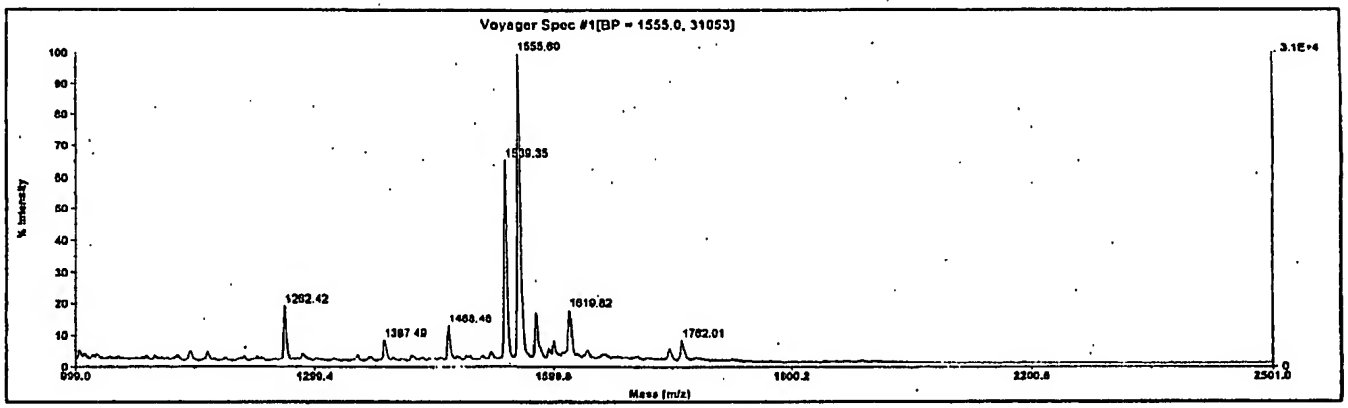


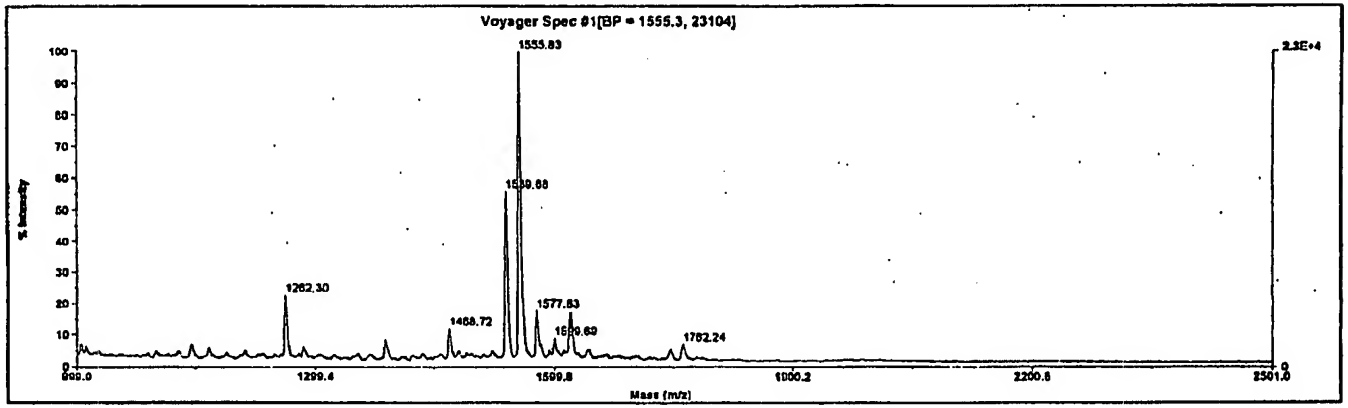
Fibirinogen 18mg/ml
Control

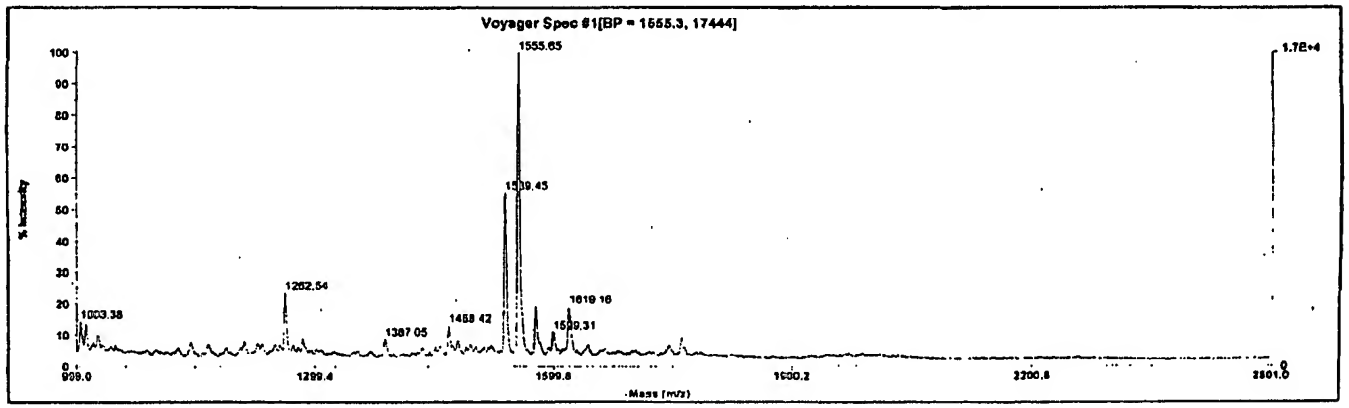




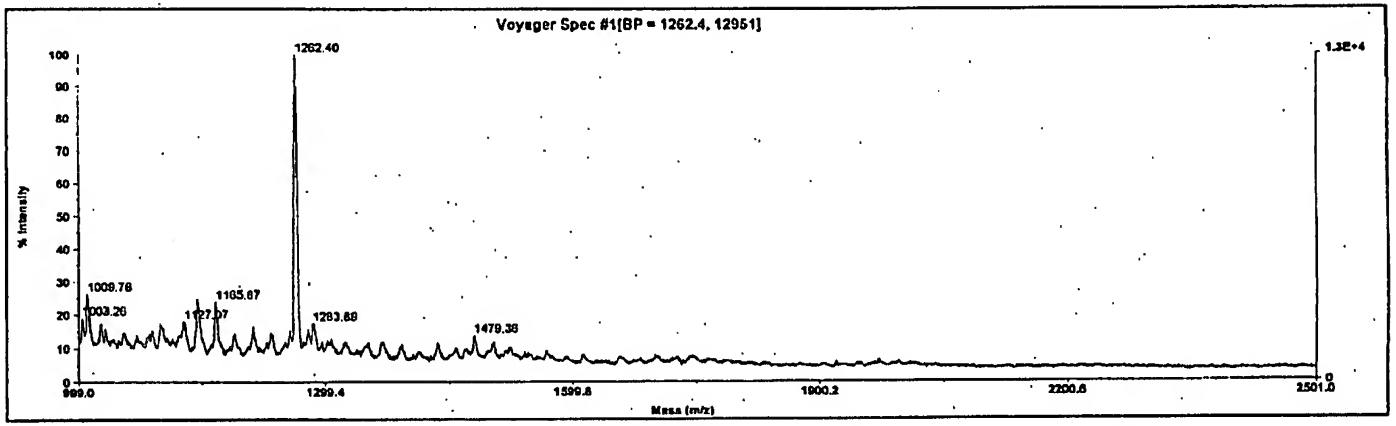
Fibirinogen 9mg/ml

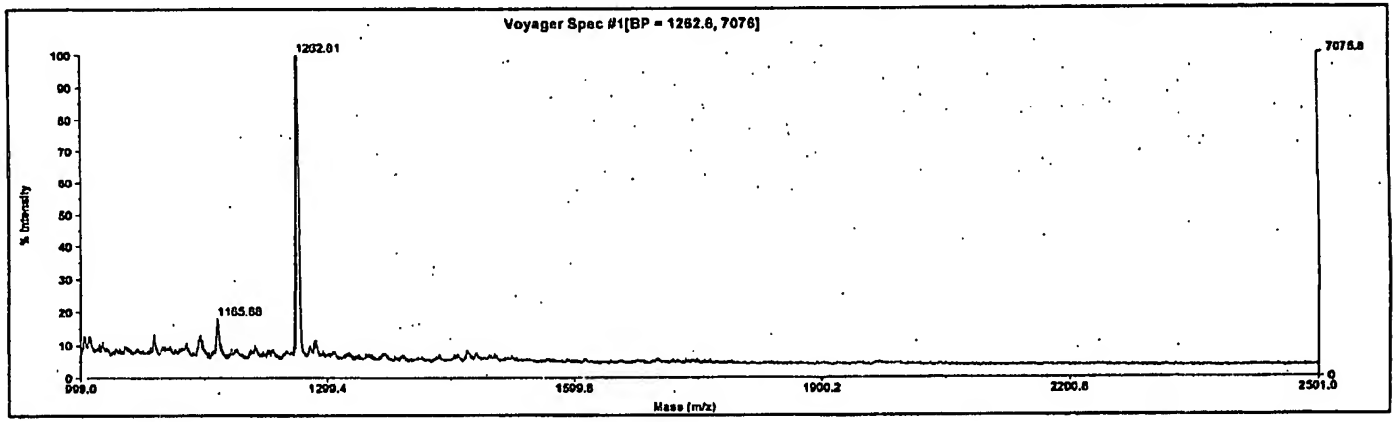




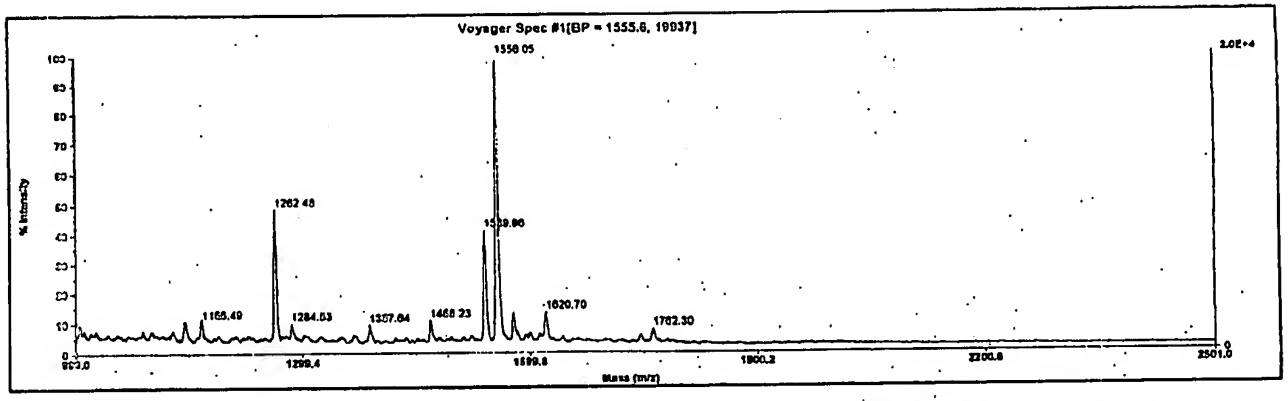


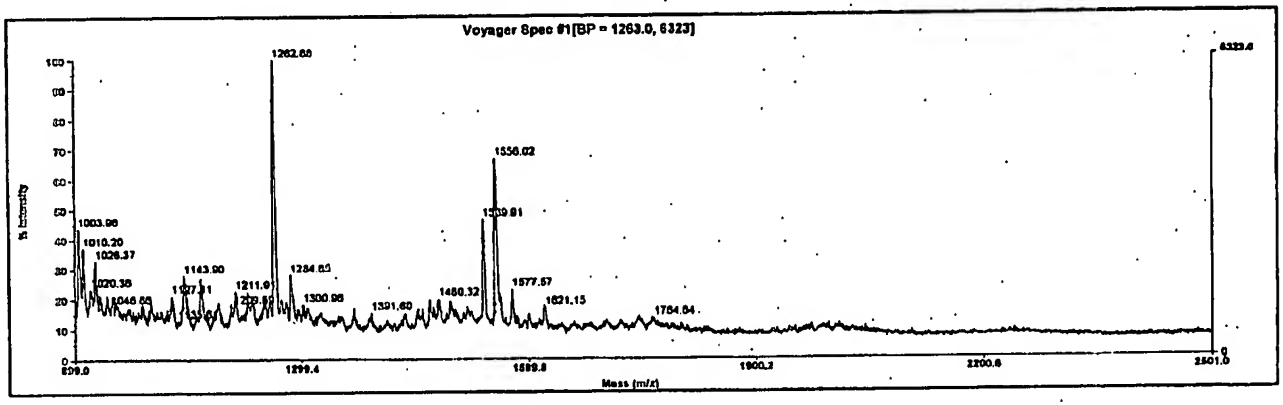
Fibrinogen 9mg/ml
Control

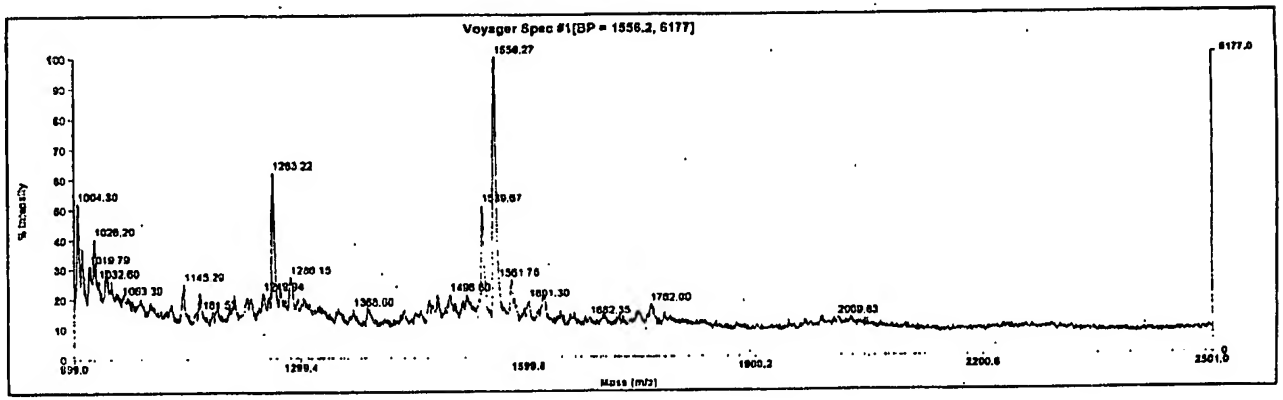




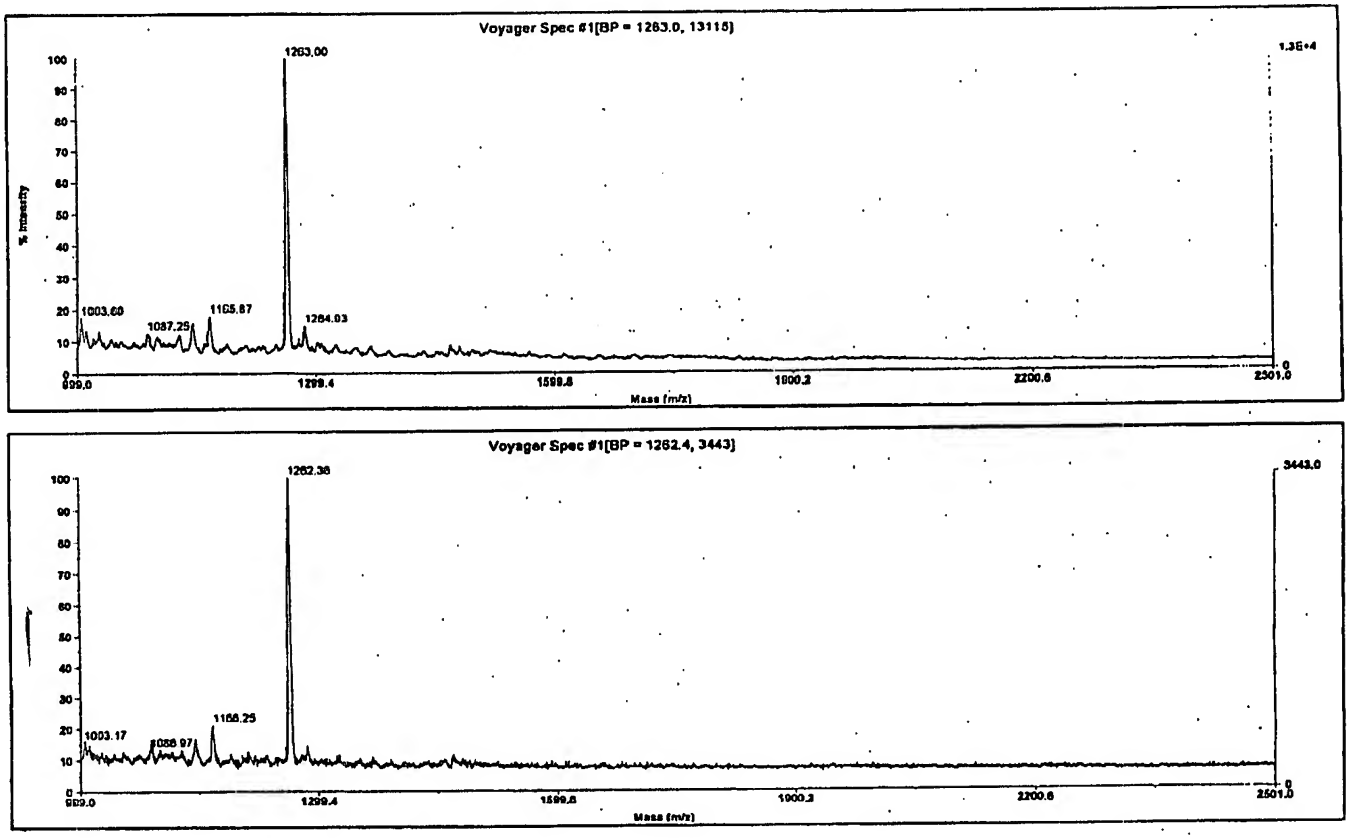
Fibrinogen 4.5mg/ml



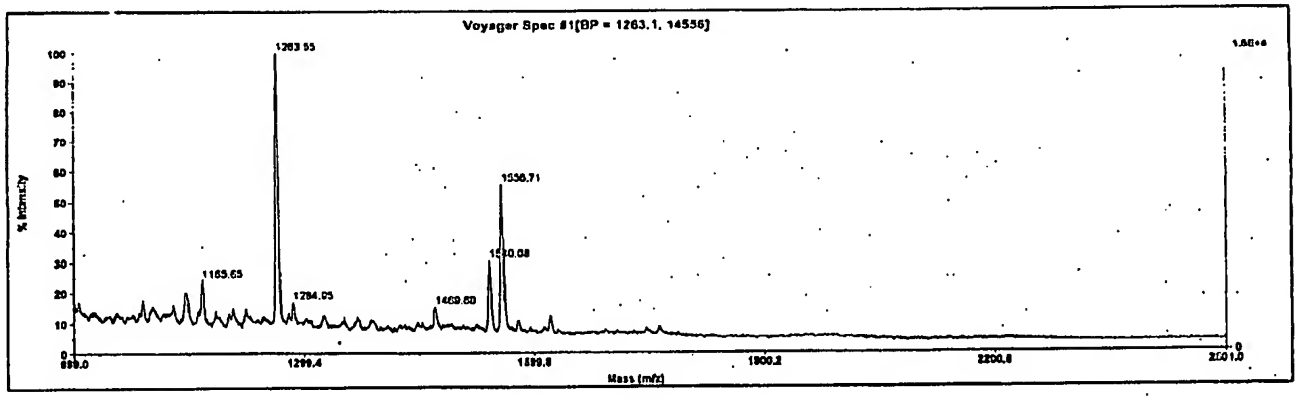


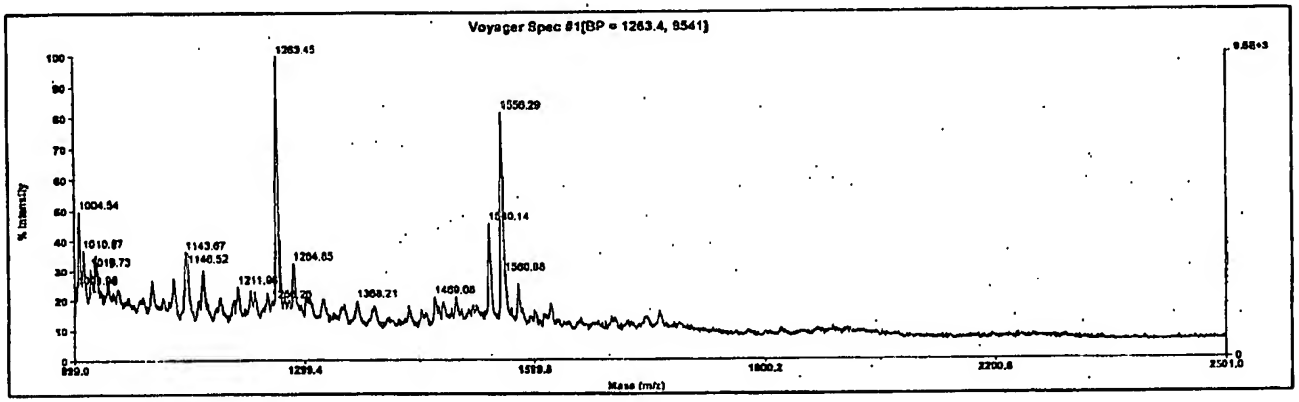


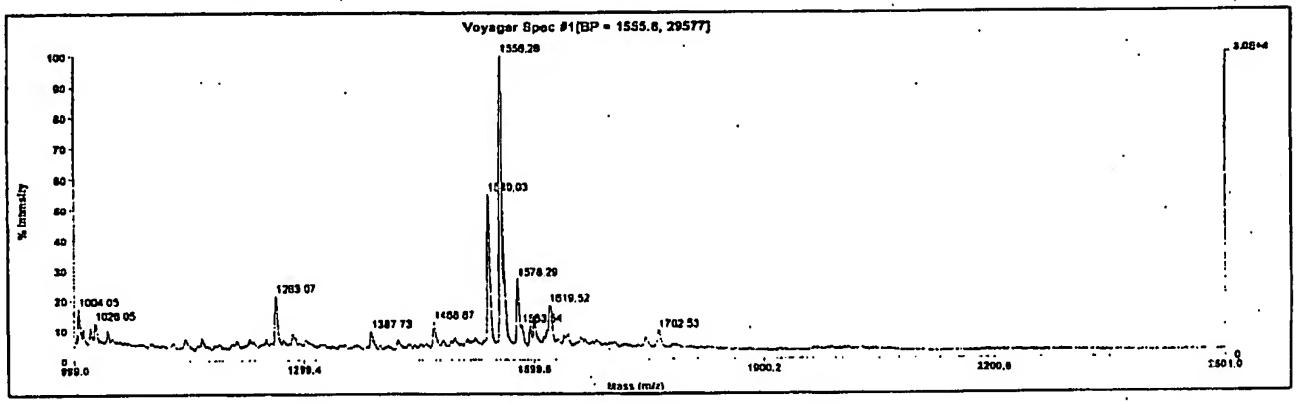
Fibrinogen 4.5mg/ml
Control



Fibrinogen 3mg/ml







Fibrinogen 3mg/ml
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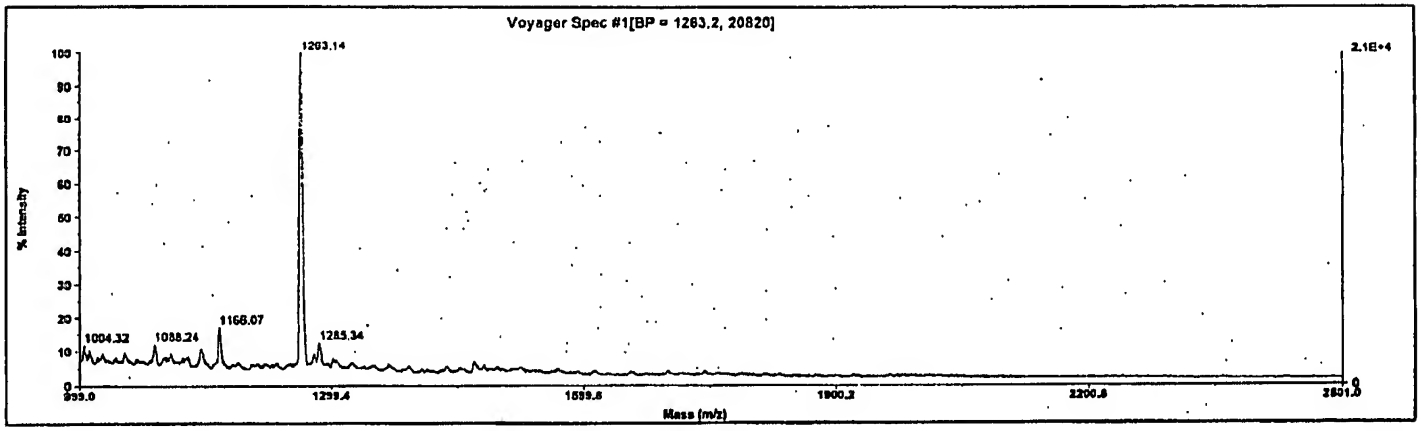
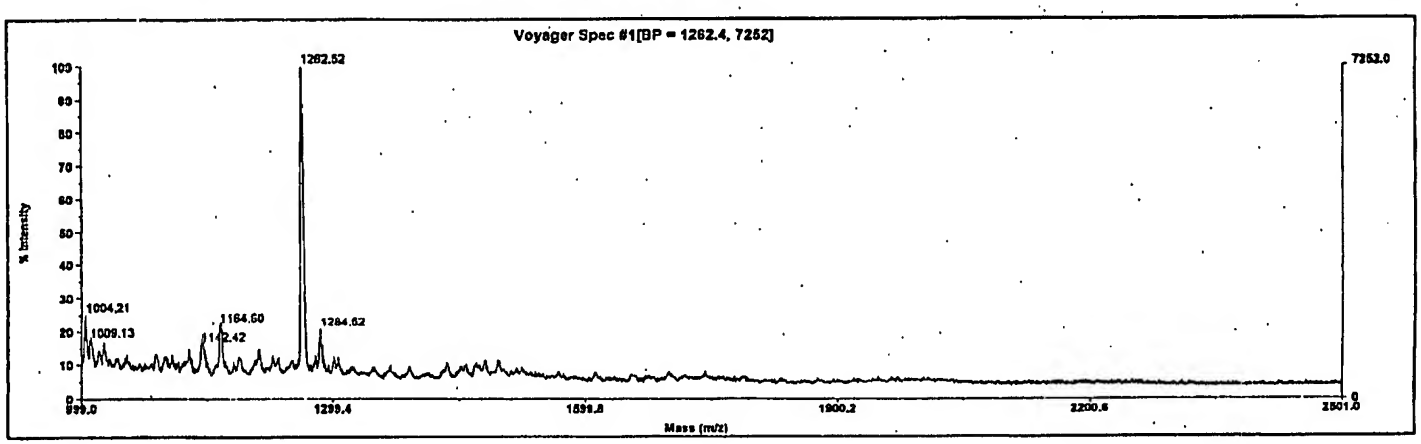
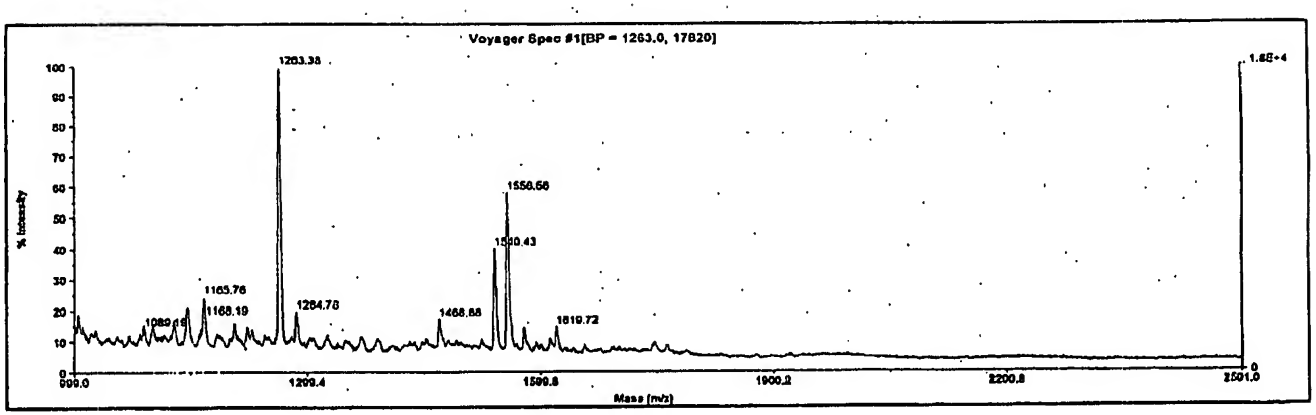
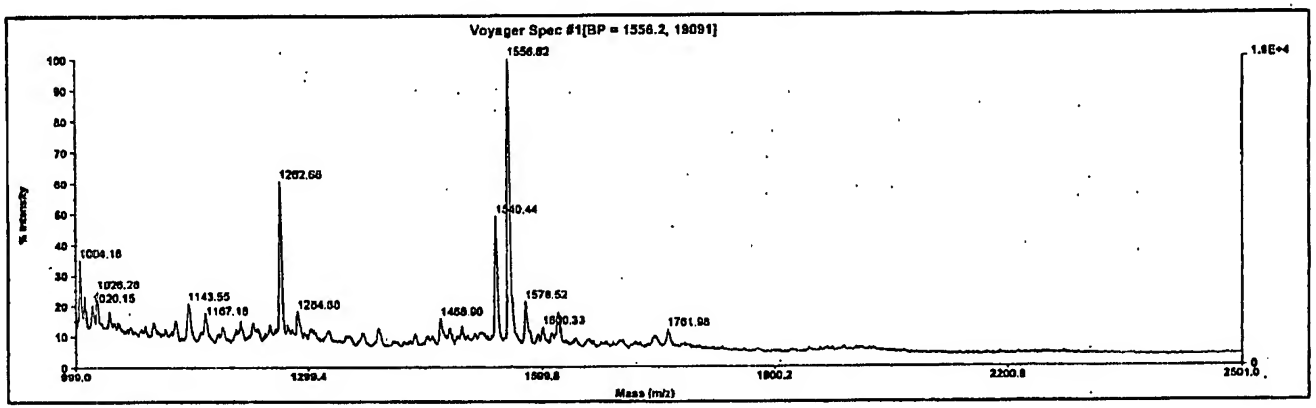
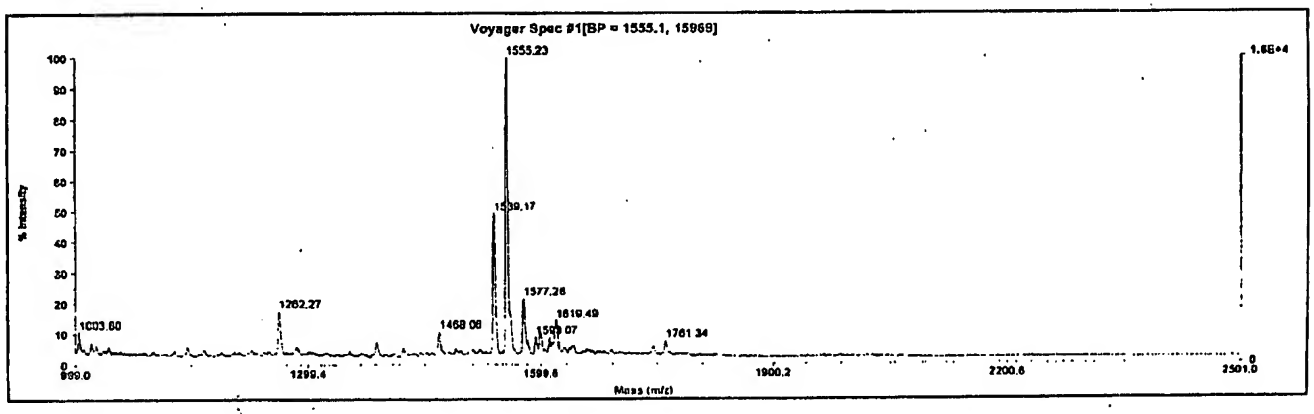




Fibrinogen 1.5mg/ml







Fibrinogen 1.5mg/ml
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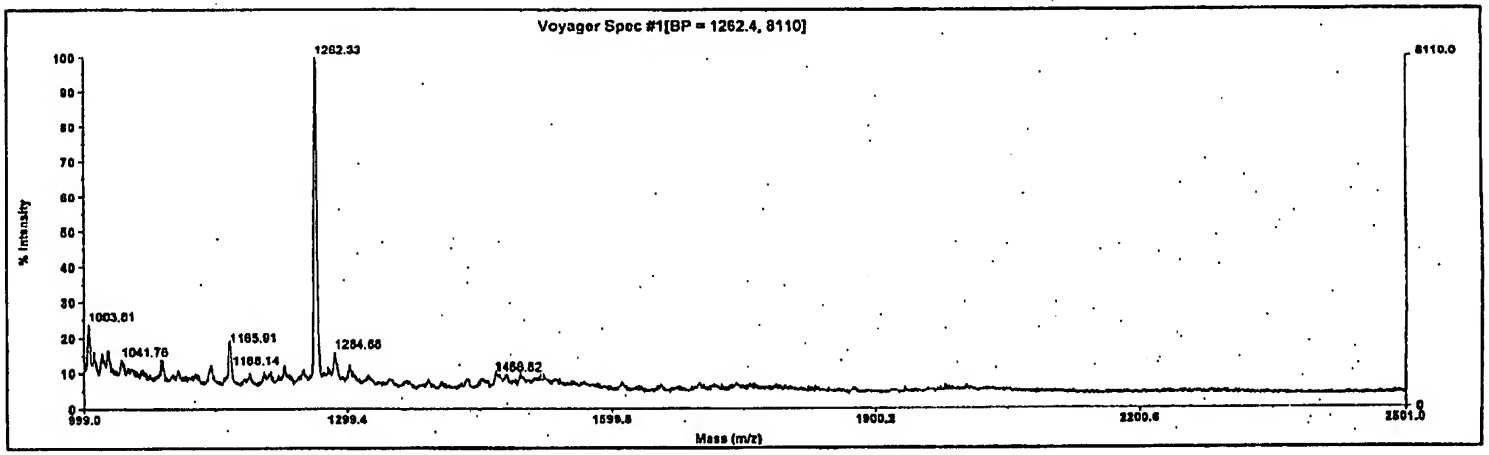
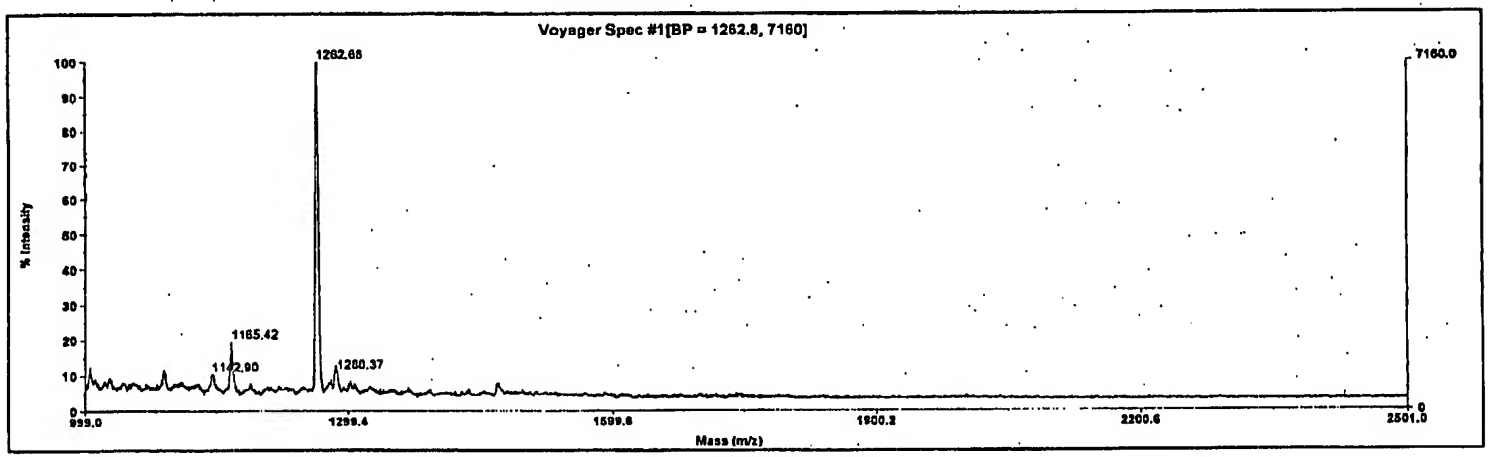
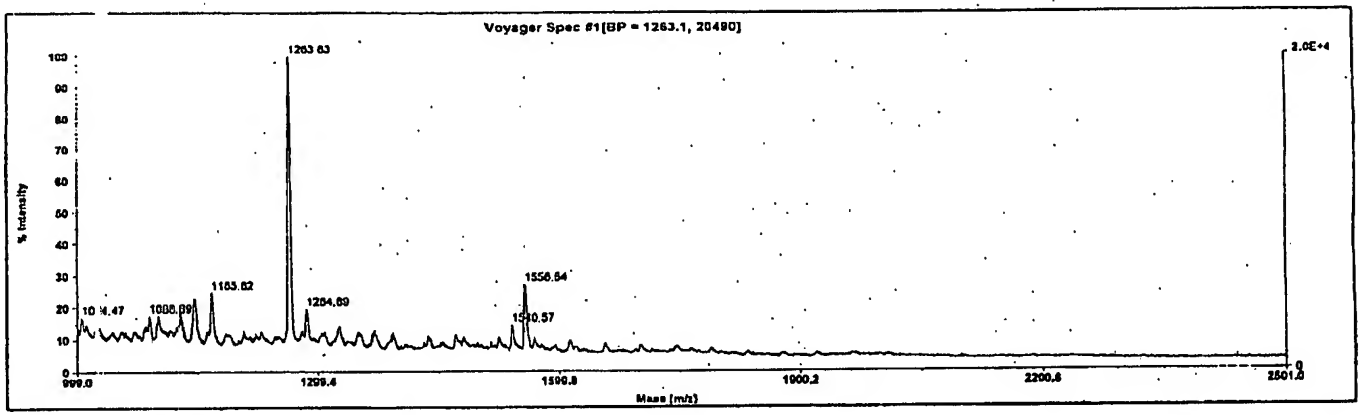
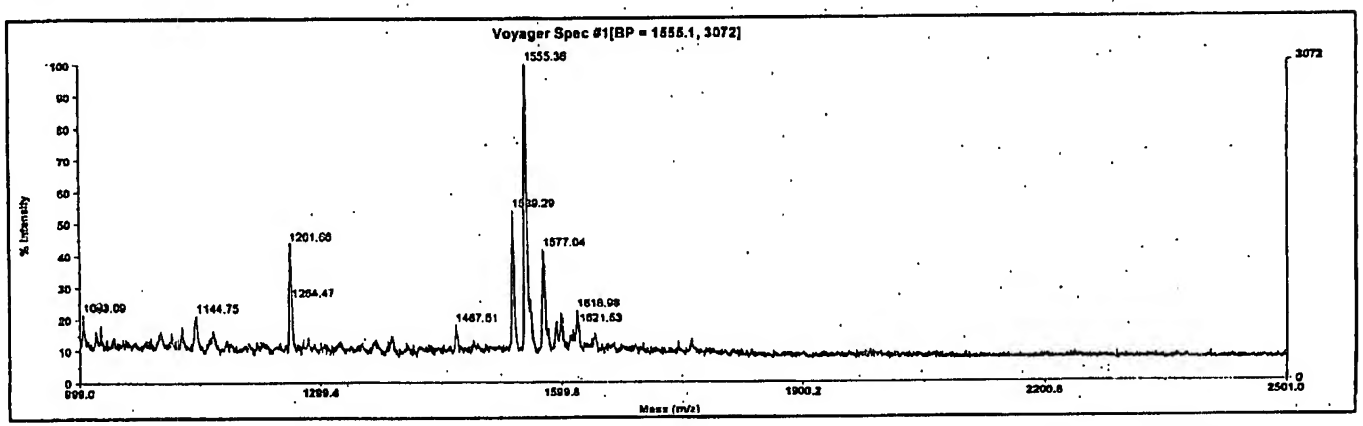
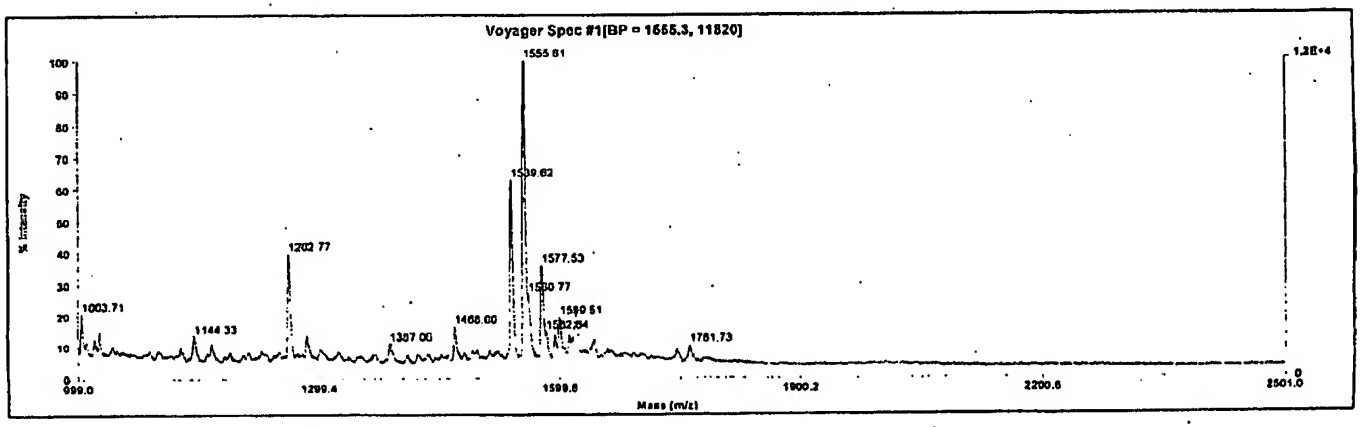




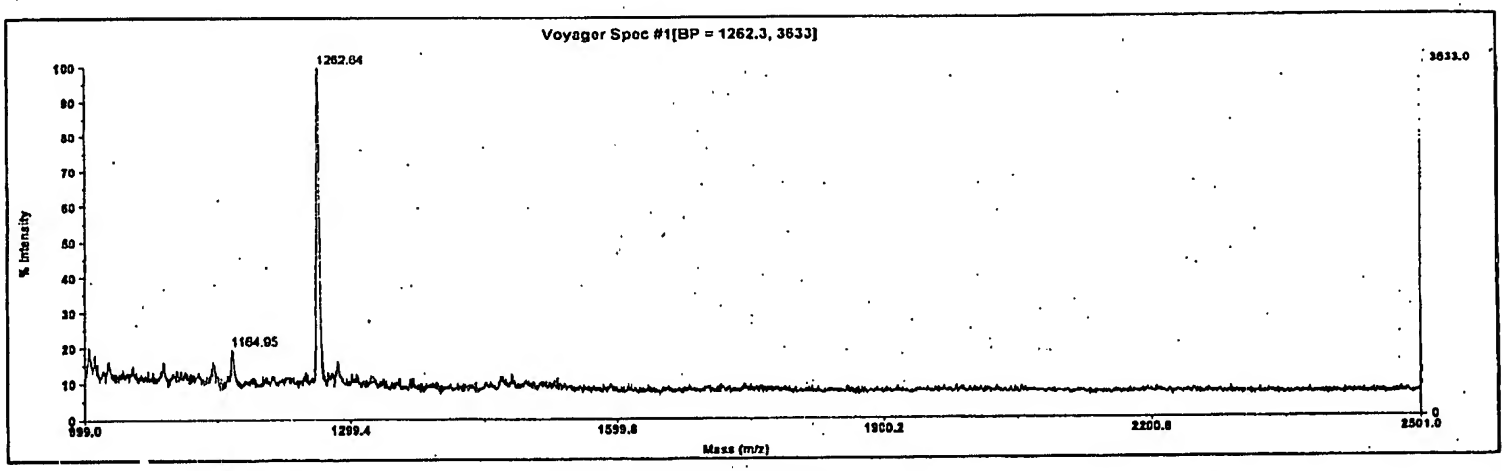
Fibrinogen 0.75mg/ml

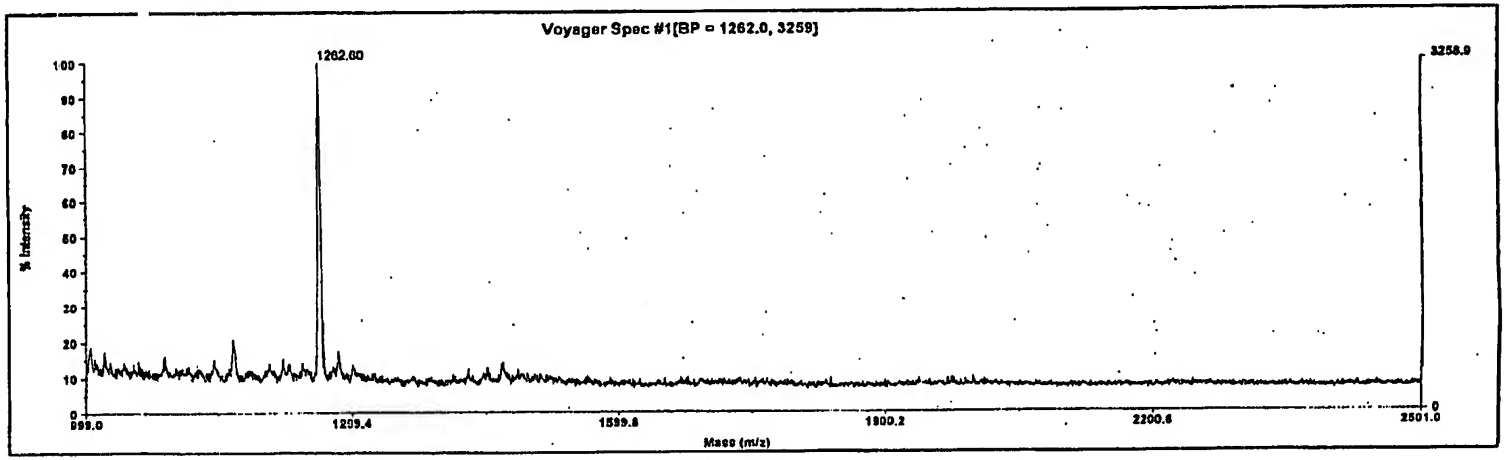






Fibrinogen 0.75mg/ml
Control





Amino Acid Calculation Results

Amino Acid Sequence

A D S G E G D F L A E G G G V R Fibrinopeptide A

N-Terminal Group: Hydrogen	**Mono Isotopic Mass:** 1535.68516	**Sequence Length:** 16
C-Terminal Group: Free Acid	**Average Mass:** 1536.5755	

Symbols	Name	Mono Mass	Average Mass	Count
Ala A	Alanine	71.03711	71.07880	2
Arg R	Arginine	156.10111	156.1876	1
Asn N	Asparginine	114.04293	114.1039	
Asp D	Aspartic Acid	115.02694	115.0886	2
Cys C	Cysteine	103.00919	103.1448	
Glu E	Glutamic Acid	129.04259	129.1155	2
Gln Q	Glutamine	128.05858	128.1308	
Gly G	Glycine	57.02146	57.0520	5
His H	Histidine	137.05891	137.1412	
Ile I	Isoleucine	113.08406	113.1595	
Leu L	Leucine	113.08406	113.1595	1
Lys K	Lysine	128.09496	128.1742	
Met M	Methionine	131.04049	131.1986	
Phe F	Phenylalanine	147.06841	147.1766	1
Pro P	Proline	97.05276	97.1167	
Ser S	Serine	87.03203	87.0782	1
Thr T	Threonine	101.04768	101.1051	
Trp W	Tryptophan	186.07931	186.2133	
Tyr Y	Tyrosine	163.06333	163.1760	
Val V	Valine	99.06841	99.1326	1

Please direct problems/questions to: Michael MacCoss

Amino Acid Calculation Results

Amino Acid Sequence				
Q G V N D N E E G F F S A R Fibrinopeptide B				
N-Terminal Group: Hydrogen		**Mono Isotopic Mass:** 1568.68552		**Sequence Length:** 14
C-Terminal Group: Free Acid		**Average Mass:** 1569.6078		
Symbols	**Name**	**Mono Mass**	**Average Mass**	**Count**
Ala A	Alanine	71.03711	71.07880	1
Arg R	Arginine	156.10111	156.1876	1
Asn N	Asparginine	114.04293	114.1039	2
Asp D	Aspartic Acid	115.02694	115.0886	1
Cys C	Cysteine	103.00919	103.1448	
Glu E	Glutamic Acid	129.04259	129.1155	2
Gln Q	Glutamine	128.05858	128.1308	1
Gly G	Glycine	57.02146	57.0520	2
His H	Histidine	137.05891	137.1412	
Ile I	Isoleucine	113.08406	113.1595	
Leu L	Leucine	113.08406	113.1595	
Lys K	Lysine	128.09496	128.1742	
Met M	Methionine	131.04049	131.1986	
Phe F	Phenylalanine	147.06841	147.1766	2
Pro P	Proline	97.05276	97.1167	
Ser S	Serine	87.03203	87.0782	1
Thr T	Threonine	101.04768	101.1051	
Trp W	Tryptophan	186.07931	186.2133	
Tyr Y	Tyrosine	163.06333	163.1760	
Val V	Valine	99.06841	99.1326	1

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